Global Change Biology (2005) **11**, 1997–2008, doi: 10.1111/j.1365-2486.2005.01006.x

Host-specific aphid population responses to elevated CO_2 and increased N availability

ERIKA A. SUDDERTH, KRISTINA A. STINSON[1] and F. A. BAZZAZ
The Biological Laboratories, Harvard University, Cambridge, MA 02138, USA

Abstract

Sap-feeding insects such as aphids are the only insect herbivores that show positive responses to elevated CO_2. Recent models predict that increased nitrogen will increase aphid population size under elevated CO_2, but few experiments have tested this idea empirically. To determine whether soil nitrogen (N) availability modifies aphid responses to elevated CO_2, we tested the performance of *Macrosiphum euphorbiae* feeding on two host plants; a C_3 plant (*Solanum dulcamara*), and a C_4 plant (*Amaranthus viridis*). We expected aphid population size to increase on plants in elevated CO_2, with the degree of increase depending on the N availability.

We found a significant $CO_2 \times N$ interaction for the response of population size for *M. euphorbiae* feeding on *S. dulcamara*: aphids feeding on plants grown in ambient CO_2, low N conditions increased in response to either high N availability or elevated CO_2. No population size responses were observed for aphids infesting *A. viridis*. Elevated CO_2 increased plant biomass, specific leaf weight, and C : N ratios of the C_3 plant, *S. dulcamara* but did not affect the C_4 plant, *A. viridis*. Increased N fertilization significantly increased plant biomass, leaf area, and the weight : height ratio in both experiments. Elevated CO_2 decreased leaf N in *S. dulcamara* and had no effect on *A. viridis*, while higher N availability increased leaf N in *A. viridis* and had no effect in *S. dulcamara*. Aphid infestation only affected the weight : height ratio of *S. dulcamara*.

We only observed an increase in aphid population size in response to elevated CO_2 or increased N availability for aphids feeding on *S. dulcamara* grown under low N conditions. There appears to be a maximum population growth rate that *M. euphorbiae* aphids can attain, and we suggest that this response is because of intrinsic limits on development time and fecundity.

Keywords: aphid performance, *Amaranthus viridis*, C_3, C_4, elevated CO_2, herbivory, *Macrosiphum euphorbiae*, nitrogen, *Solanum dulcamara*

Received 16 December 2004; revised version received 24 March 2005; accepted 30 March 2005

Introduction

The effects of elevated CO_2 and increased N deposition on insect herbivory will depend on how these global change factors alter the nutritional status of the plant tissues consumed by herbivores. Global climate models predict that atmospheric CO_2 concentrations will rise to between 540 and 970 ppm by 2100, from 280 ppm in pre-industrial times (Wigley & Raper, 2001). Industrial nitrogen (N) production, agricultural run-off, and land conversion have all resulted in increased terrestrial and aquatic N concentrations (Vitousek *et al.*, 1997). The physiology of plants grown at elevated CO_2 levels is altered in several ways, leading to reduced plant host quality, including decreased leaf N content, increased concentrations of phenolics (Whittaker, 1999), and increases in foliage water content (Coviella & Trumble, 1999). In high CO_2, the ratio of carbon to nitrogen (C : N) in foliage generally increases because of higher photosynthetic rates and faster growth (Watt *et al.*, 1993). Plants that utilize C_3 photosynthesis typically show larger growth responses to elevated CO_2 than C_4 plants, resulting in larger increases in foliage C : N ratios in C_3 plants (Bazzaz, 1990).

Correspondence: Erika A. Sudderth, Department of Organismic & Evolutionary Biology, Harvard University, Biological Laboratories, 16 Divinity Ave., Cambridge, MA 02138, USA, fax +1 617 496 5854, e-mail: sudderth@fas.harvard.edu

[1] Present address: Harvard Forest, PO Box 68, Petersham, MA 01366, USA.

Nitrogen is an important limiting resource for phytophagous insects (Mattson, 1982). Thus any factor altering the supply of C or N in plant tissue has the potential to modify herbivore consumption and fitness (Diaz *et al*., 1998). Aphids may be particularly sensitive to environmental factors that cause changes in nutrient transport within a plant because they feed directly on phloem sap (Jansson & Ekbon, 2002). Phloem sap is primarily composed of sugar compounds (Lalonde *et al*., 2003) and contains a relatively low concentration of amino acids ($\sim 20\%$), while the composition of aphid bodies is approximately 50% amino acids (Sandstrom & Moran, 1999). Therefore, aphids must significantly concentrate N from their food sources to add biomass. Although it is widely recognized that aphids are sensitive to the nutrient profiles of their plant hosts (Sandstrom & Moran, 2001; Karley *et al*., 2002), it is not clear how aphid performance will be modified by plant physiological responses to simultaneous changes in atmospheric CO_2 and N availability.

Sap feeders are the only feeding guild in which positive responses to elevated CO_2 have been observed (Whittaker, 1999). Numerous studies examining the response of aphid species to elevated CO_2 have found that aphid populations may variably increase, decrease, or be unaffected by elevated CO_2. The aphid species that have been shown in some experiments to respond positively to elevated CO_2, such as *Sitobion avenae*, *Aulacorthum solani*, and *Myzus persicae*, are polyphagous feeders (Awmack *et al*., 1996, 1997; Bezemer *et al*., 1998, 1999; Whittaker, 1999; Hughes & Bazzaz, 2001). However, not all polyphagous aphid species respond positively or consistently to elevated CO_2. Although *A. solani* had increased fecundity and faster development when reared on plants under elevated CO_2 in one experiment (Awmack *et al*., 1997), the same species showed no response (Awmack & Harrington, 2000), or a negative response (Hughes & Bazzaz, 2001), when population parameters were measured. Further supporting these findings, Awmack *et al*. (2004) observed that the growth and fecundity of individual *Cepegillettea betulaefoliae* aphids did not predict the population growth rate in this species. A study of the effects of *M. euphorbiae* infestation of *S. dulcamara* in response to elevated temperature and CO_2 (Flynn *et al*., 2005) found that the populations of this generalist aphid species did not increase in elevated CO_2. In a review of herbivore responses to elevated CO_2, Coviella *et al*. (1999) and Lawton (2000) conclude that the effect of elevated CO_2 will be specific to each insect–plant system.

While many researchers have concluded that aphid responses to elevated CO_2 will be idiosyncratic, Newman *et al*. (2003) present a model concluding that aphid population responses may be predicted based on interactions between soil N fertility, the aphids' N requirement, and the density-dependent response in winged morph production. The model predicts that aphid populations may increase, decrease, or remain unchanged in response to CO_2 enrichment, and the response depends on N dynamics. For a given aphid species with a fixed N requirement, increased N will increase aphid population size under elevated CO_2. In contrast, aphid species with a high N requirement and a strong density-dependent response in winged morph production may have smaller populations in elevated CO_2. The predictions of the Newman *et al*. model have not been tested because the combined influence of CO_2 and N on both plant and aphid performance have rarely been measured simultaneously (but see Docherty *et al*., 1997; Diaz *et al*., 1998).

Numerous experiments have shown that elevated CO_2 and N both can alter plant performance and leaf chemistry. Fajer *et al*. (1992), observed a significant interaction between the effects of elevated CO_2 and nutrient availability on the reproductive weight of *Plantago lanceolata*. Starch and sugar concentrations in four grass species were observed to respond in a species-specific manner to elevated CO_2, depending on the availability of soil nutrients (Goverde *et al*., 2002). Kerslake *et al*. (1998) found that elevated CO_2 did not affect the growth response of *Calluna vulgaris*, while increased N supply resulted in increased shoot growth and a lower C : N ratio. The performance of a leaf-feeding herbivore (*Operophtera brumata*) corresponded to the observed plant responses, with elevated CO_2 having no effect, and N increasing larval development, growth rate, and pupal weight. In a study of plant allocation to defense compounds, Coviella *et al*. (2002) concluded that that availability of N determined plant allocation to defensive compounds in response to elevated CO_2, and subsequently altered herbivore performance. These results show the importance of nutrient availability in determining plant response to elevated CO_2, and may explain in part why aphid responses to elevated CO_2 have been inconsistent. Most experiments studying aphid performance in response to elevated CO_2 do not vary or report the N concentrations used (Newman *et al*., 2003).

Despite the evidence for interactions between CO_2 and N effects on plant physiology, only a few studies have investigated how these factors might influence insect herbivore response to global change. Most of these studies examine leaf-chewing herbivores feeding on tree species (Kinney *et al*., 1997; Hattenschwiler & Schafellner, 1999; Henn & Schopf, 2001; Saxon *et al*., 2004) or shrubs (Johnson & Lincoln, 1991; Kerslake *et al*., 1998). We know of only two studies that have investigated the combined effects of CO_2 and N on aphid performance (Docherty *et al*., 1997; Diaz *et al*., 1998), and neither observed an effect of elevated CO_2 on aphid performance. Docherty *et al*. (1997) found that neither atmospheric CO_2 nor fertilizer level affected the population size of beech aphids. Diaz *et al*., (1998) found that N fertilization increased snail and aphid fecundity while CO_2 had no effect on herbivore performance. Given that the studies which have found significant increases in aphid performance in response to elevated CO_2 did not vary N growth conditions (Awmack *et al*., 1996, 1997; Bezemer *et al*., 1998, 1999; Whittaker, 1999; Hughes & Bazzaz, 2001), the role of N availability in determining aphid response to elevated CO_2 requires further study.

In this study, we investigated the importance of soil N availability in modifying aphid response to elevated CO_2 by examining the performance of a generalist phloem-feeding insect herbivore (*Macrosiphum euphorbiae*) feeding on plants grown under a factorial combination of two CO_2 and two N treatments. We chose one C_3 (*Solanum dulcamara*) and one C_4 (*Amaranthus viridis*) plant species, in which we expected large and small growth responses, respectively, to elevated CO_2. We tested the hypothesis that aphid abundance would increase in elevated CO_2, but only under conditions of sufficient soil N availability. Based on the predicted relative growth responses of the plant species, we expected aphids to show a greater increase in performance under elevated CO_2 and high N on the C_3 species (*S. dulcamara*) than on the C_4 species (*A. viridis*). We also expected larger aphid populations to cause greater reductions in plant performance. We discuss our results in terms of the general predictions made by the Newman *et al*. (2003) model of aphid responses to elevated CO_2 and N fertilization. Our experiment was completed before the Newman *et al*. model was published, and was, therefore, not designed to mimic the model parameters and precisely test its predictions. However, there are few available data sets that test the interactive effects of elevated CO_2 and N on aphid population response. To our knowledge, this study is the first attempt to use experimental data to test the model-generated hypothesis that aphid response to elevated CO_2 may be predicted based on interactions with N availability.

Materials and methods

Study species

M. euphorbiae (Thomas), utilizes Rosa species as a primary host, and is highly polyphagous on secondary hosts. This species is an important crop pest, acting as a vector for over 45 viruses, including beet yellow net and bean leaf roll (Blackman & Eastop, 2000). Despite the importance of *M. euphorbiae* as a generalist agricultural pest, the response of this species to elevated CO_2 has only been investigated in one other study (Flynn *et al*., 2005). Adult aphids were collected in Boston, Massachusetts, in the summer of 2001 and reared continuously in growth chambers on *S. dulcamara* L. and *A. viridis*, at 23 °C and ambient CO_2. The experimental colonies were founded from one individual, and therefore consisted of genetically identical clones. The rearing plants were closely inspected upon removal from the colonies, and no signs of disease were ever observed.

S. dulcamara L. (bittersweet nightshade) is a weedy, rhizomatous C_3 perennial with a worldwide distribution. It possesses a climbing or scrambling growth form, and thrives in a variety of habitats, from waterlogged and shaded sites, to dry and fully exposed sites (Pegtel, 1985). *A. viridis* (slender amaranth or pigweed) is a pantropical C_4 weed found in warm temperate regions, with an obscure origin (USDA, *Germplasm Resources Information Network*). We chose the plant species for this experiment based on their expected growth responses to elevated CO_2 because of their respective photosynthetic physiologies. *S. dulcamara* was chosen because it is a C_3 plant that has demonstrated significant growth responses to elevated CO_2, while *A. viridis* is a C_4 plant species with smaller growth responses to elevated CO_2 (E. A. Sudderth, personal observation). Both species were expected to show significantly enhanced growth under high N conditions. In addition, both species had been observed to support large populations of *M. euphorbiae* aphids in the greenhouse.

Plant growth conditions

S. dulcamara. In June 2001, seeds were planted in trays filled with 1 : 1 : 1 sand : peat moss : vermiculite, and placed in six environmental growth chambers (Conviron, Pembina, ND, USA), with three maintained at ambient (370 ppm) and three maintained at elevated (750 ppm) atmospheric CO_2. The growth chambers' temperatures were 25 °C day and 20 °C night, with a 16 : 8 day : night light regime. Daytime light levels in the chambers averaged $220\,\mu mol\,m^{-2}\,s^{-2}$ over the course of the experiment. This light level approximates the understory light environment where *S. dulcamara* often grows. Nutrient treatments began upon germination. Half of the seedlings from each CO_2 treatment were watered with a modified Hoagland's solution containing either 1 mmol or 3 mmol N, respectively. In preliminary experiments, the low N level chosen had been observed to produce adequate growth, while the high N treatment significantly increased plant growth.

Two weeks after germination 128 seedlings (16 replicates × 2 CO_2 levels × 2 N levels × 2 infestation levels) were transplanted into 2.5 L pots with the same soil mixture as described above. Plants from each combination of CO_2 and nutrient treatment were randomly assigned to one of three chambers maintained at the same CO_2 level (ambient or elevated) in which the plants were germinated (six chambers were used in total). The chambers were arranged in three blocks of two adjacent chambers each, to account for possible spatial variability in microenvironmental factors. To ensure ample water availability, we provided equal amounts of water ($\sim$350 mL every 2 days) to all plants in the experiment. Plants were watered and fertilized every few days via two parallel automatic watering systems (The Drip Store, Escondido, CA, USA), installed in all six chambers used in the experiment. The nutrient treatments described above were delivered via two continuous feed injection systems (Dosatron International, Bordeaux, France), one for each nutrient treatment. In the fifth week after germination, two adult apterous aphids were introduced onto half of the plants in each $CO_2 \times N$ treatment. All plants were caged in light-weight netting supported by collapsible wire frames. Watering and fertilization continued via the automatic watering system until harvest.

A. viridis

In July 2002, seeds were planted in 1 : 1 : 1 sand : peat moss : vermiculite, and placed in four environmentally controlled greenhouse zones, two maintained at 370 ppm and two maintained at 750 ppm atmospheric CO_2, to germinate. The zones were maintained at 25 °C day and 20 °C night, with a 16 : 8 day : night light regime. When ambient light levels dropped below 600 $\mu mol\, m^{-2}\, s^{-2}$ during the daytime period, light levels were supplemented with 1000 W high-density discharge lights suspended over the benches. Daytime light levels in the zones averaged 730 $\mu mol\, m^{-2}\, s^{-2}$ during the course of the experiment. Nutrient treatments began upon germination via the automatic watering system as described above. A water-soluble fertilizer (MiracleGro EXCEL Cal-Mag 15-5-15, Scotts, Marysville, Ohio, USA) mixed to deliver 25 ppm N was used for both nutrient treatments. The high N treatment received an additional 50 ppm ammonium nitrate, for a total treatment level of 75 ppm N.

Two weeks after germination, 128 total seedlings were transplanted into 2.5 L pots using the same soil mixture described above. Plants from each combination of CO_2 and N treatment were randomly assigned to one of two zones maintained at each CO_2 level, corresponding to the CO_2 level at which the plant were germinated (four total zones were used). The plants were watered and fertilized every few days, as described for *S. dulcamara*. Two adult apterous *M. euphorbiae* aphids were introduced onto half of the plants in each $CO_2 \times N$ treatment in the third week after germination. The plants were covered with custom lightweight nets constructed from paint-strainer material (DC May, DC May INC, Charlotte, NC, USA), and supported with custom collapsible wire frames (Pacific Wire, Pacific Wire Supply INC, Escondido, CA, USA).

Data collection

Plant heights for both experiments were recorded on the dates of seedling transplant, aphid introduction, and total harvest (2, 3, and 4 weeks after germination, respectively). Plants from both experiments were harvested in the fourth week after the aphid introductions. Because of time constraints, a subsample of the infested plants was harvested (9–15 replicates). Aphids were brushed into a container containing 95% ethanol with a fan-shaped paintbrush and stored at 5 °C until they were counted. The total leaf area of each harvested plant was measured with an LI-3100 leaf area meter (Li-Cor, Li-Cor, Lincoln, NE, USA). After measuring the leaf area, one fully expanded leaf (approximately the eighth leaf from the apex) was removed and dried separately for C : N analysis (7–10 replicates of each treatment were analyzed). The rest of the plant was separated into the leaf, stem, and root portions, and dried at 65 °C for biomass analysis.

Dry weight of the leaves, stems and roots, leaf C : N ratios, and the aphid population size were determined for each plant. The weight : height ratio was calculated as aboveground biomass (g dry weight) divided by the plant height (cm) to determine how the architecture of the plants responded to the treatments. The total number of aphids from each harvested plant was counted, and then the aphids were dried and the total weight of the population from each plant was determined. C : N analysis of the leaf material was performed by the University of Georgia Soil Ecology Lab using a C/H/N analyzer (NA1500, Carlo Erba Strumentazione, Milan, Italy).

Statistical analyses

The main effects of CO_2, N, and infestation on plant performance (aboveground biomass, leaf area, specific leaf weight, weight : height ratio, C : N ratio, and leaf N content) were tested by four-factor ANOVA according to the model below. We used a split–split plot design with CO_2 and block (pair of adjacent chambers) as the main effects, N as the subplot effect, and aphid presence or absence as the sub-subplot effect (ANOVA, JMP, SAS Institute, SAS Campus Drive, Cary, NC, USA).

ANOVA model:

$$X_{ijklm} = \mu + C_i + B(C)_{j(i)} + N_k + CN_{ik} + NB(C)_{kj(i)} + I_l + CI_{il} + IB(C)_{lj(i)} + NIB(C)_{klj(i)} + e_{m(ijkl)},$$

where C is the CO_2 treatment, B the block, N the nitrogen treatment, and I the Infestation. X_{ijklm} represents the average plant response at CO_2 level i in block j, N level k, and infestation level l. $e_{m(ijkl)}$ represents the error because of the smaller scale differences between samples because of variability within blocks.

The effects of CO_2 and N on aphid populations were tested by three-factor ANOVAs using a split–plot design with CO_2 and block as the main effects and N as the subplot effect. Data were transformed if necessary (ln, arc sin, or box–cox) to satisfy assumptions of normality and variance homogeneity for the ANOVA tests. Box–cox transformations were performed using the Factor Profiling/Box–Cox Y transformation function in JMP. ANOVA results are shown in Tables 1 and 2. Linear regression was used to determine if aphid population size, individual aphid weight, or the number of aphids cm^{-2} leaf area depended on the C : N ratio or N content of plant leaves.

Results

Aphid performance

The average population size of *M. euphorbiae* aphids feeding on *S. dulcamara* (Fig. 1a) was approximately an order of magnitude greater than that of aphids feeding on *A. viridis* (Fig. 1b). There were no significant effects of the treatments on aphids feeding on *A. viridis*. There was a significant N response ($P = 0.007$) and a significant $CO_2 \times N$ interaction ($P = 0.012$) for aphids feeding on *S. dulcamara* (Table 1); aphid population size in response to elevated CO_2 varied, depending on plant N availability. Aphid population size on low N *S. dulcamara* plants increased in elevated CO_2, while aphid population size on high N plants did not increase under elevated CO_2 (Fig. 1a). CO_2 and N did not significantly affect the average aphid weight in either experiment (Figs 1c, d). Neither aphid population size, individual aphid weight, nor the number of aphids cm^{-2} leaf area depended on the C : N ratio or N content of plant leaves (results not shown).

Plant performance

Elevated CO_2 significantly increased the aboveground biomass (Fig. 2a) and specific leaf weight (Fig. 3a) of the C_3 plant, *S. dulcamara*, however, no significant responses to elevated CO_2 were observed for the C_4 plant, *A. viridis* (Figs 2b and 3b). Increased N availability significantly increased aboveground biomass (Figs 2a, b), weight : height ratio (Figs 2c, d), and leaf area (Figs 3c, d), of both plant species at both ambient and elevated CO_2, with or without aphid infestation (Table 2). The only significant effect of aphid infestation on plant performance was an increase in the weight : height ratio of *S. dulcamara* plants in response to aphid infestation (Fig. 2c).

Leaf N content

The C : N ratio of *S. dulcamara* leaves significantly increased in elevated CO_2, and was not affected by N availability (Table 2). In contrast, CO_2 did not affect the C : N ratio and high N decreased the C : N ratio in the leaves of *A. viridis* (Figs 4a, b). As expected, leaf N content showed the inverse response from the C : N ratio in both species (Figs 4c, d).

Table 1 Summary of ANOVA results for effects of elevated CO_2 and increased nitrogen (N) on aphid performance

		Solanum dulcamara (C_3)			*Amaranthus viridis* (C_4)		
Measurement	Treatment	df	*F*-ratio	Prob > *F*	df	*F*-ratio	Prob > *F*
Aphid number/plant	CO_2	1	0.780	0.427	1	0.188	0.706
	N	**1**	**21.193**	**0.007**	1	1.519	0.334
	$CO_2 \times N$	**1**	**16.580**	**0.012**	1	4.944	0.146
Mass per aphid	CO_2	1	1.422	0.296	1	1.443	0.350
	N	1	1.243	0.326	1	3.538	0.196
	$CO_2 \times N$	1	0.094	0.774	1	1.114	0.399

Significant *P*-values ($P < 0.05$) are shown in bold face type.

Table 2 Summary of ANOVA results for effects of elevated CO_2, increased nitrogen (N), and aphid infestation on plant performance and leaf N content

		Solanum dulcamara (C_3)			*Amaranthus viridis* (C_4)		
Measurement	Treatment	df	*F*-ratio	Prob > *F*	df	*F*-ratio	Prob > *F*
Aboveground mass	CO_2	**1**	**11.292**	**0.028**	1	0.028	0.882
	N	**1**	**100.330**	**0.001**	**1**	**33.678**	**0.028**
	Infestation	1	0.002	0.971	1	0.865	0.451
	CO_2 × N	1	0.591	0.485	1	0.026	0.887
	CO_2 × infestation	1	0.122	0.744	1	0.034	0.871
	N × infestation [CO_2]	2	0.968	0.454	2	0.525	0.656
Leaf area	CO_2	1	0.064	0.812	1	1.443	0.352
	N	**1**	**133.868**	**< 0.001**	**1**	**1020.615**	**0.001**
	Infestation	1	0.057	0.824	1	0.512	0.548
	CO_2 × N	1	0.069	0.806	1	1.144	0.395
	CO_2 × infestation	1	1.815	0.249	1	0.883	0.446
	N × infestation [CO_2]	2	2.194	0.227	2	6.021	0.142
Specific leaf weight	CO_2	**1**	**19.251**	**0.012**	1	0.426	0.581
	N	1	3.661	0.128	1	5.033	0.153
	Infestation	1	1.135	0.346	1	0.158	0.992
	CO_2 × N	1	0.710	0.447	1	0.236	0.402
	CO_2 × infestation	1	1.090	0.355	1	6.332	0.596
	N × infestation [CO_2]	2	3.276	0.143	2	0.344	0.370
g mass cm^{-1} $height^{-1}$	CO_2	1	5.864	0.073	1	0.004	0.957
	N	**1**	**162.974**	**< 0.001**	**1**	**369.708**	**0.003**
	Infestation	**1**	**21.011**	**0.010**	1	1.603	0.331
	CO_2 × N	1	3.532	0.132	1	2.950	0.226
	CO_2 × infestation	1	0.569	0.492	1	3.074	0.220
	N × infestation [CO_2]	2	1.934	0.258	2	0.066	0.938
C : N	CO_2	**1**	**29.022**	**0.006**	1	0.3858	0.597
	N	1	0.497	0.519	**1**	**162.675**	**0.006**
	Infestation	1	1.738	0.255	1	0.007	0.940
	CO_2 × N	1	0.121	0.746	1	0.172	0.718
	CO_2 × infestation	1	0.171	0.700	1	9.819	0.083
	Nit × infestation [CO_2]	2	0.918	0.469	2	0.479	0.676
% leaf N	CO_2	**1**	**31.651**	**0.005**	1	11.483	0.070
	N	1	0.427	0.549	**1**	**201.081**	**0.004**
	Infestation	1	2.838	0.164	1	0.365	0.602
	CO_2 × N	1	0.012	0.917	1	0.007	0.942
	CO_2 × infestation	1	0.725	0.440	1	13.277	0.058
	N × infestation [CO_2]	2	0.489	0.646	2	0.362	0.734

Significant *P*-values ($P < 0.05$) are shown in bold-face type, and treatments which have significant effects in both experiments are shaded.

Discussion

Aphid performance

The performance of *M. euphorbiae* aphids was hypothesized to increase when feeding on plants grown under elevated CO_2 and high N availability. Because growth stimulation by CO_2 is generally greater in C_3 than in C_4 plants, we expected aphid performance to increase more in aphids feeding on the C_3 plant, *S. dulcamara*, than on the C_4 plant, *A. viridis*. Aphids did not respond according to our predictions on either plant species. Aphids feeding on *A. viridis* had small populations and did not show any significant responses to the environmental treatments. We found a significant CO_2 × N interaction for the population size of *M. euphorbiae* aphids feeding on *S. dulcamara*; population size increased in response to higher N availability at ambient CO_2, but showed no response to N at elevated CO_2. Aphid population size increased in elevated CO_2 on *S. dulcamara* plants grown under low N, but not on plants grown under high N (Fig. 1a).

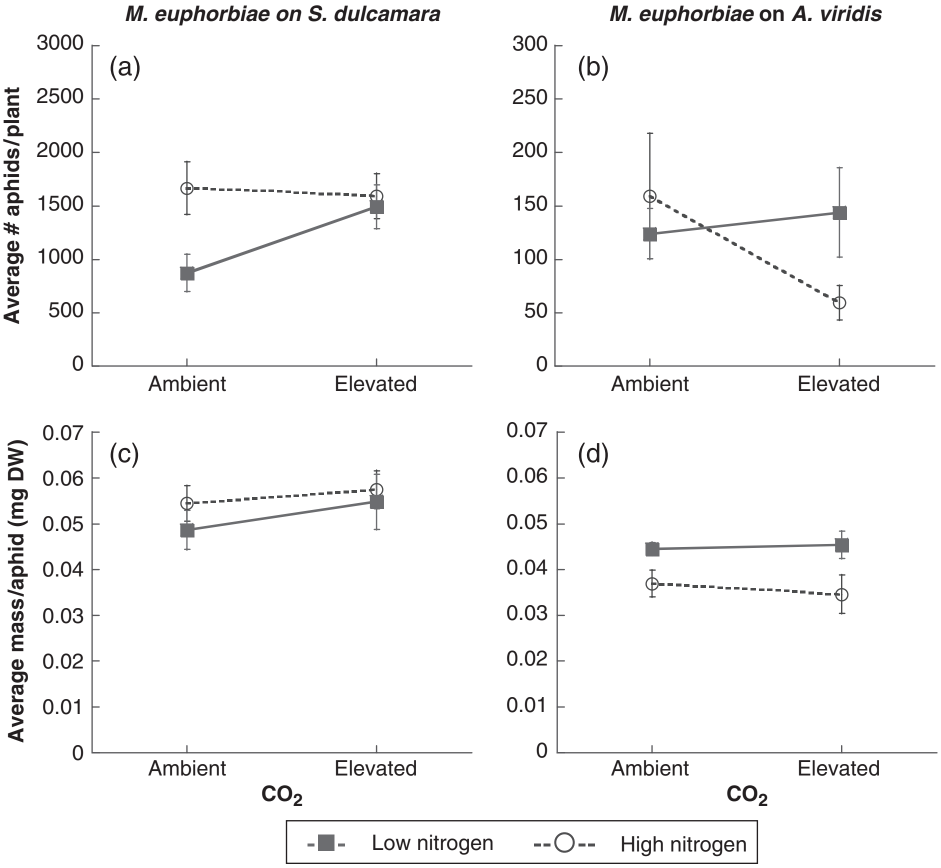

Fig. 1 Performance of *Macrosiphum euphorbiae* aphids infesting *Solanum dulcamara* (C_3) or *Amaranthus viridis* (C_4) plants (mean ± 1 SE). (a) Aphid population size on *S. dulcamara*, (b) aphid population size on *A. viridis*, (c) average individual aphid weight on *S. dulcamara*, and (d) average individual aphid weight on *A. viridis*. Note the different axis scales for the aphid population size graphs.

The Newman *et al*. (2003) model describing the influence of enhanced $CO_2 \times N$ interactions on aphid population dynamics predicts that aphid population size will be greater under elevated CO_2 with the following conditions: N inputs are high and the aphid species has a low N requirement and a weak density-dependent response in winged morph production. In addition, as soil fertility rises relative to the N requirement of the aphid species, aphids become less limited by N and more limited by other processes such as density. Moreover, adding more N to the soil increases the likelihood that aphid populations will be larger under elevated CO_2, regardless of the aphids' N requirement or the strength of its density-dependent responses (Newman *et al*., 2003).

We are not aware of any available data for the N requirement or density dependence of alate (winged) aphid morph production for *M. euphorbiae*. However, based on our observations and a study by other authors of *M. euphorbiae* response to various petunia fertilization regimes (Jansson & Ekbon, 2002), we can estimate these parameters relative to those used in the Newman *et al*. (2003) model. In our experiment, despite very high aphid density on the apical meristems and senescing leaves of *S. dulcamara*, we observed little alate production (data not shown). Therefore, lacking direct data, we infer that *M. euphorbiae* aphids have a weak density-dependent response of alate morph production. The intrinsic rate of increase (r_m) of *M. euphorbiae* aphids was found to be higher on petunia plants grown under 4.6 mmol N than on plants grown under 3.9 mmol N (Jansson & Ekbon, 2002). Because the N level of 3.9 mmol is higher than the N levels used in the model, and an increase in N fertilization to 4.6 mmol increases aphid performance, we infer that *M. euphorbiae* aphids have a high N requirement relative to the estimate for pea aphids used in the Newman *et al*. model.

Based on the above estimates for *M. euphorbiae* N requirements and density-dependent alate production, we conclude that our experimental parameters correspond to high N input, high aphid N requirement, and weak aphid density-dependent response in the

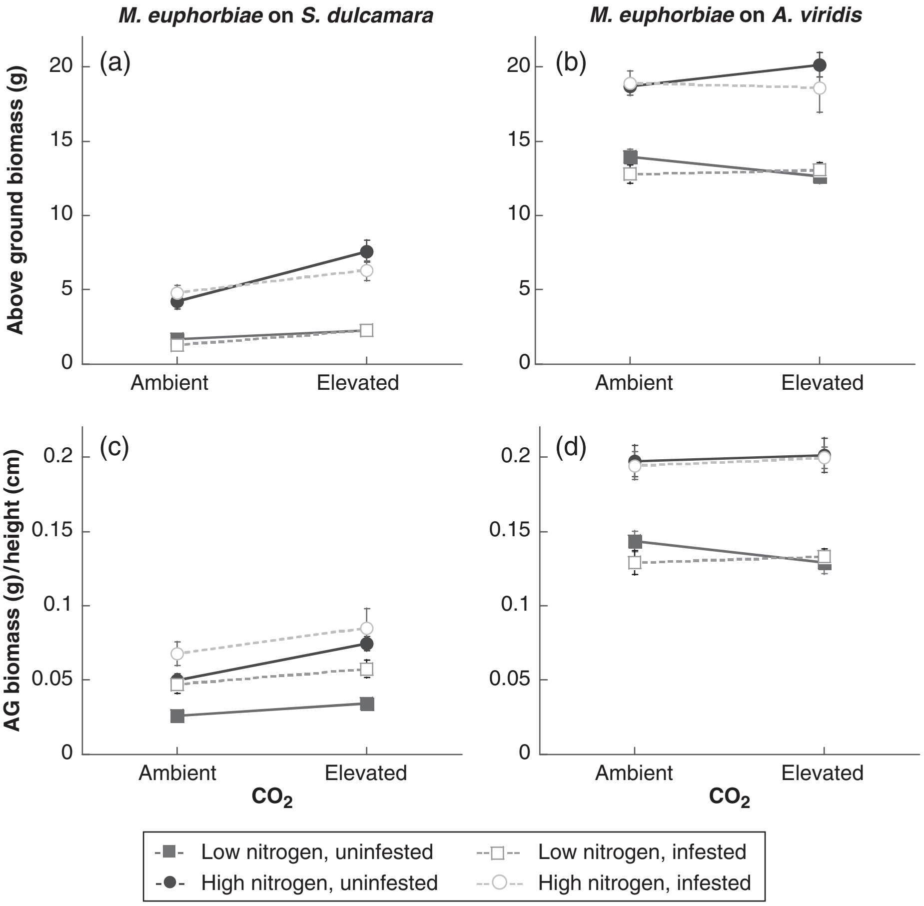

Fig. 2 Performance of *Solanum dulcamara* (C_3) and *Amaranthus viridis* (C_4) plants grown under different conditions of CO_2, nitrogen, and aphid herbivory (mean ± 1 SE). (a) *S. dulcamara* aboveground biomass, (b) *A. viridis* aboveground biomass, (c) grams of aboveground biomass cm^{-1} plant height for *S. dulcamara*, and (d) grams of aboveground biomass per cm plant height for *A. viridis*.

Newman *et al*. model. Our results agreed in part with the model. At ambient CO_2, high N increased aphid population size. This response would be predicted by the model if *M. euphorbiae* was limited by our low N treatment. Even though our low N treatments were high compared with levels used in the model, the aphids may still have been limited by N if they had a high N requirement. Aphid population size also increased in response to elevated CO_2 on plants grown under low N. This result implies that elevated CO_2 somehow alters plant physiology in a manner that affects aphid population growth in a similar manner to increased N fertilization.

Our results disagreed with the model prediction that adding more N to the system will be more likely to increase aphid population size at elevated CO_2. Aphid population size did not increase in elevated CO_2 under high N, only under low N. Other factors likely limited aphid population growth once a maximum growth rate was achieved. The Newman *et al*. model states that density may limit aphid population growth as soil fertility rises relative to aphid N requirement. Density may have limited aphid population growth in our system, but *M. euphorbiae* did not respond by increased alate production.

There are differences between the treatments in our study and the parameters of the Newman *et al*. model. A calculation based on an estimate of the liters of water received by each plant shows that our low N treatment corresponds to $\sim 0.015\,\mathrm{kg\,N\,m^{-2}}$ every 15 days. We cannot directly compare our continuous feed N treatments, in which drainage through the pots can leach N from the system, to the $\mathrm{kg\,N\,m^{-2}}$ applied to the soil in the Newman model. However, according to our calculation, our low N treatment level is 50% higher than the highest N level included in the model. Therefore, in extrapolating the model results to our data, we may overlook potential nonlinear or inhibitory effects of the higher N input. In addition, we introduced two aphids to each plant and harvested

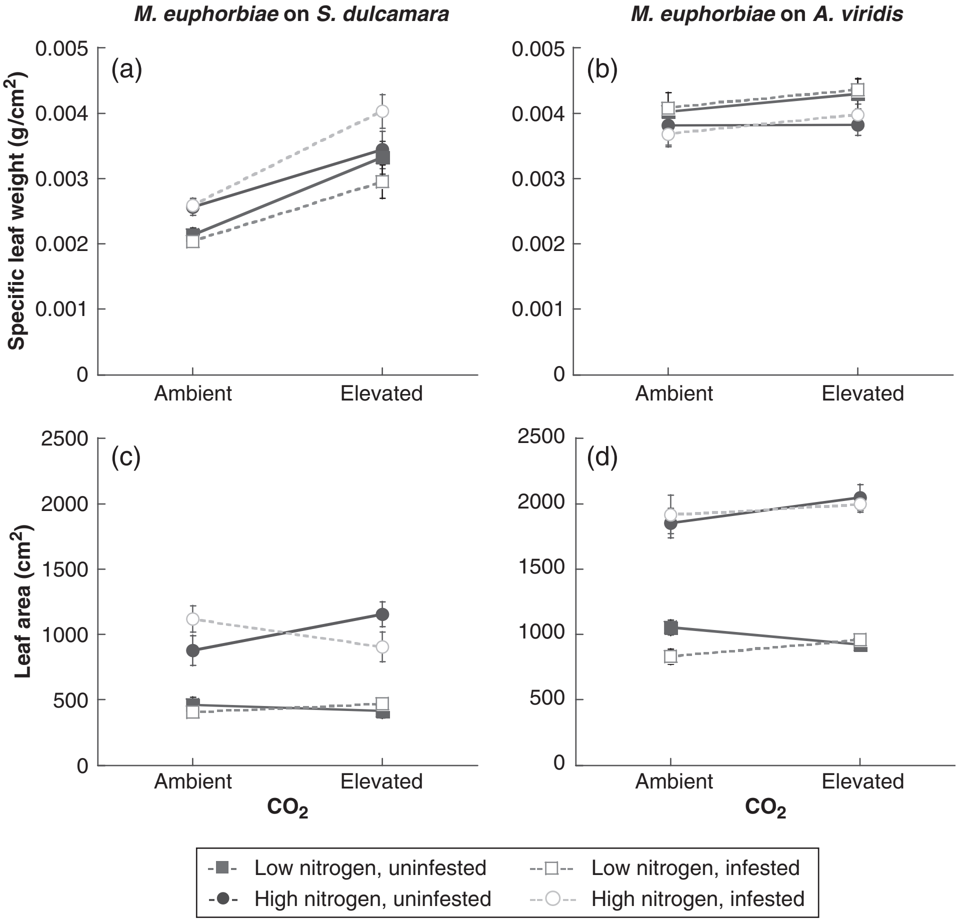

Fig. 3 Leaf characteristics of *Solanum dulcamara* (C_3) and *Amaranthus viridis* (C_4) grown under different conditions of CO_2, nitrogen, and aphid herbivory (mean ± 1 SE). (a) *S. dulcamara*-specific leaf weight, (b) *A. viridis* specific leaf weight, (c) *S. dulcamara* leaf area, and (d) *A. viridis* leaf area.

the experiment in the fourth week after aphid introduction, while the model starts with five initial aphids and models population growth over 70 days.

We observed large differences in the average population size of *M. euphorbiae* aphids feeding on *S. dulcamara* and *A. viridis* grown under different conditions of atmospheric CO_2 and soil N availability. Final aphid population size was on average an order of magnitude smaller on *A. viridis* than on *S. dulcamara* (Fig. 1a). Although this result provides limited support for the theory that insect herbivores tend to perform better on C_3 than on C_4 plants (Barbehenn & Bernays, 1992), our experiments did not explicitly test this hypothesis. The dramatic difference in final population size for *M. euphorbiae* aphids feeding on *S. dulcamara* vs. *A. viridis* could be explained if distinct factors limited aphid population growth in each situation. A particular characteristic of *A. viridis*, such as defensive chemistry or low nutrient phloem contents, may have prevented rapid population growth rates, and had a stronger effect than the environmental treatments. For *S. dulcamara*, there may be a maximum population growth rate that can be achieved by *M. euphorbiae* under any combination of environmental conditions.

We hypothesize that aphid population growth rate is limited by intrinsic factors such as a minimum development time per aphid or a maximum number of offspring that each aphid could produce, regardless of how favorable the environmental growth conditions are. These intrinsic factors would impose an asymptotic limit on the population growth rate, such that once the maximum growth rate is achieved, the aphids would be unable to respond to an improvement in the environmental growth conditions. This maximum growth rate may have been achieved in all environmental treatments except the ambient CO_2, low N treatment (Fig. 1a). Once the maximum growth rate was achieved in either high N or elevated CO_2, the growth rate could not increase any further, even with the addition of a second beneficial factor.

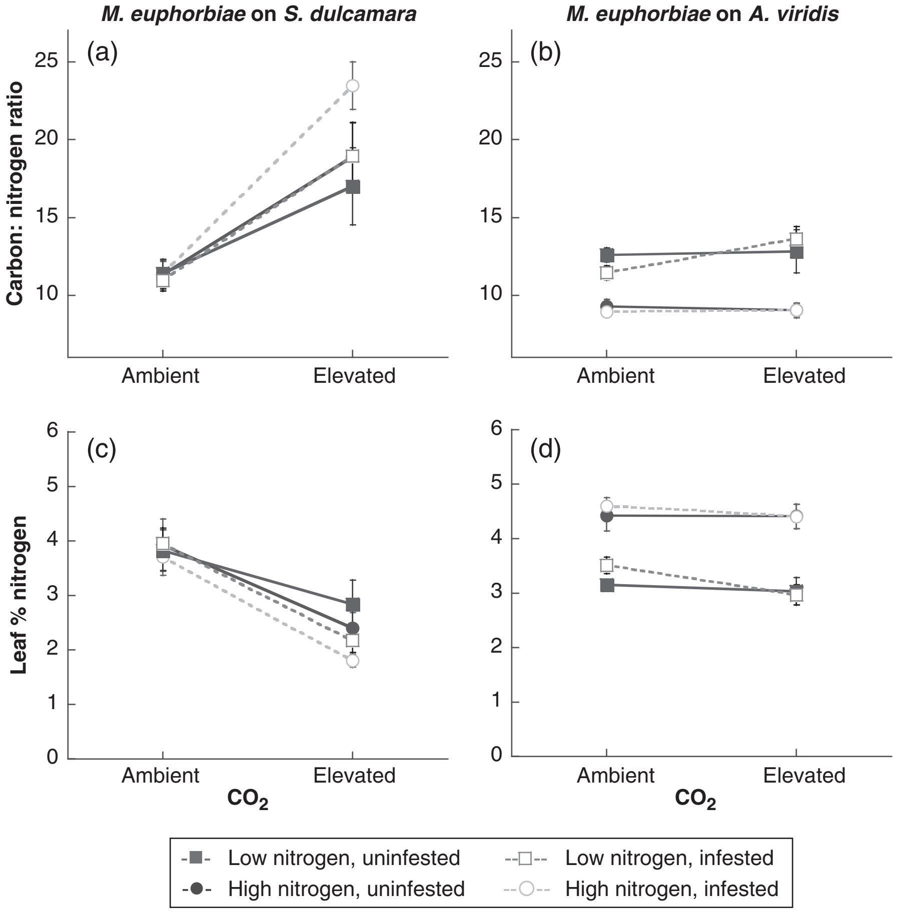

Fig. 4 Leaf nitrogen response of *Solanum dulcamara* (C_3) and *Amaranthus viridis* (C_4) plants grown under different conditions of CO_2, nitrogen, and aphid herbivory (mean ± 1 SE). (a) C : N ratio for *S. dulcamara* leaves, (b) C : N ratio for *A. viridis* leaves, (c) % nitrogen content for *S. dulcamara* leaves, and (d) % nitrogen content for *A. viridis* leaves.

Consistent with other studies (Hughes & Bazzaz, 2001), we found that the aphid population responses to our treatments of elevated CO_2 and increased N availability did not correspond to changes in the C : N ratios of the leaf tissue. It is not known if the N content of the phloem sap corresponds to foliar N levels in *S. dulcamara* and *A. viridis*. Aphid performance has been shown to be most influenced by the ratio of sucrose to amino acids in the phloem sap, rather than the C : N ratio (Abisgold *et al.*, 1994). Future studies would benefit by investigating the interaction between N fertilization and the ratio of sugars to amino acids in phloem sap in response to elevated CO_2.

The relationship between sugar and amino acid concentrations in the phloem sap and aphid–symbiont interactions should also be explored. Aphids harbor *Buchnera* and other bacterial symbionts that provide the aphids with essential amino acids that are limiting in phloem sap, and cannot be synthesized by the aphids (Wilkinson & Ishikawa, 2001). Aphids can therefore be buffered from variation in the supply of the amino acids by their symbiotic bacteria (Douglas *et al.*, 2001; Karley *et al.*, 2002). Our results show that the mechanism via which aphids respond to the nutritional status of their plant hosts is not yet understood. Elucidating the role of aphid symbionts in mediating the response of aphid performance to global change variables such as elevated CO_2 and N availability may explain the variable aphid responses observed across numerous experiments.

Plant performance

Elevated CO_2 and N availability had the expected effect of increasing plant biomass in the C_3 plant *S. dulcamara*. CO_2 and N both significantly affected several plant performance variables, with CO_2 causing a slightly greater number of significant effects compared with N availability in *S. dulcamara*. *A. viridis* responded differently to the experimental treatments. Elevated

CO_2 did not alter any plant or aphid performance variables, while N significantly changed five out of the six variables reported. These results are expected based on the photosynthetic pathways of each plant species. In general, C_3 plants respond positively to elevated CO_2 while C_4 plants typically show smaller growth responses (Bazzaz, 1990).

The growth architecture of *S. dulcamara* and *A. viridis* was significantly altered by increased N availability. The weight : height ratio of both species increased when grown in high N. A higher weight : height ratio indicates that a plant is investing more biomass in each centimeter of height, either by increased thickness of its plant parts or by increasing branch and leaf production. The only plant performance variable affected by aphid infestation was the weight : height ratio of *S. dulcamara*. The weight : height ratio increased indicating that *S. dulcamara* was able to increase branch and leaf production (personal observation), even under extremely high infestation rates (600–1700 aphids per plant). The aphids tended to feed in large numbers on the apical meristems of the plants, leading to damaged apical tissue. Apical meristem damage typically induces a branching response in plants because of the loss of apical dominance (Huhta *et al*., 2003). The observed effects of severe aphid infestation on plant architecture, but not on aboveground biomass, highlight the importance of including allometry in plant growth analysis.

Conclusions

Our results show that elevated CO_2 and increased N availability can increase aphid population size. However, there appears to be a maximum population growth rate that *M. euphorbiae* aphids can attain, and we hypothesize that this response is because of intrinsic limits on development time and fecundity. Even large aphid populations did not affect most measures of plant performance, contrasting with observations from similar studies. For example, Hughes *et al*. (2001) found a consistent negative effect of aphid infestation on final plant biomass. These results demonstrate that different aphid species may have distinct population level responses to the same plant species. N availability affected more plant characteristics than either atmospheric CO_2 concentration or aphid infestation in *A. viridis*. In the *S. dulcamara* experiment, CO_2 was more important than N in determining the response of plant characteristics, but N still had several significant effects. In *S. dulcamara*, where aphid infestation increased the weight : height ratio, increased N availability reduced the negative influence of aphid herbivory on plant growth. Our results demonstrate the important influence that the selected N level can have on the outcome of an experiment.

The observed response of *M. euphorbiae* populations to increased N under ambient and elevated CO_2 highlights the need for a better mechanistic model to further our understanding of the interactions between nutrient uptake, phloem transport, aphid feeding, symbiont response, and limits to aphid population growth. N levels vary significantly in agricultural and natural ecosystems. Some species of aphid herbivores have been shown to respond positively to elevated CO_2, which may increase plant damage under future atmospheric conditions. In addition, N levels are expected to continue to increase because of N deposition and agricultural run-off. Understanding the role of N in determining how aphid populations will respond to elevated CO_2 remains an important challenge in global change research.

Acknowledgements

We thank Dan Flynn for assistance with the experiments, Amity Wilczek, Kristin Lewis, Tristram Seidler, and Kelly Wolfe-Bellin for advice on experimental design and data analysis, and Leslie Hughes and three anonymous reviewers for comments on the manuscript. This research was supported by a NSF grant (#9903808) to F. A. B.

References

Abisgold JD, Simpson SJ, Douglas AE (1994) Nutrient regulation in the pea aphid *Acyrthosiphon pisum* – application of a novel geometric framework to sugar and amino-acid consumption. *Physiological Entomology*, **19**, 95–102.

Awmack CS, Harrington R (2000) Elevated CO_2 affects the interactions between aphid pests and host plant flowering. *Agricultural and Forest Entomology*, **2**, 57–61.

Awmack CS, Harrington R, Leather SR (1997) Host plant effects on the performance of the aphid *Aulacorthum solani* (Kalt.) (Homoptera: Aphididae) at ambient and elevated CO_2.. *Global Change Biology*, **3**, 545–549.

Awmack CS, Harrington R, Leather SR *et al*. (1996) The impacts of elevated CO_2 on aphid–plant interactions. *Aspects of Applied Biology*, **45**, 317–322.

Awmack CS, Harrington R, Lindroth RL (2004) Aphid individual performance may not predict population responses to elevated CO_2 or O_3. *Global Change Biology*, **10**, 1414–1423.

Barbehenn RV, Bernays EA (1992) Relative nutritional quality of C_3 and C_4 grasses for a graminivorous leptidopteran, *Paratrytone melane* (Hesperiidae). *Oecologia*, **92**, 97–103.

Bazzaz FA (1990) The response of natural ecosystems to the rising global CO_2 levels. *Annual Review of Ecology and Systematics*, **21**, 167–196.

Bezemer TM, Jones TH, Knight KJ (1998) Long-term effects of elevated CO_2 and temperature on populations of the peach potato aphid *Myzus persicae* and its parasitoid *Aphidius matricariae*. *Oecologia*, **116**, 128–135.

Bezemer TM, Knight KJ, Newington JE *et al*. (1999) How general are aphid responses to elevated atmospheric CO_2? *Annals of the Entomological Society of America*, **92**, 724–730.

Coviella CE, Stipanovic RD, Trumble JT (2002) Plant allocation to defensive compounds: interactions between elevated CO_2 and nitrogen in transgenic cotton plants. *Journal of Experimental Botany*, **53**, 323–331.

Coviella CE, Trumble JT (1999) Effects of elevated atmospheric carbon dioxide on insect–plant interactions. *Conservation Biology*, **13**, 700–712.

Diaz S, Fraser LH, Grime JP *et al*. (1998) The impact of elevated CO_2 on plant–herbivore interactions: experimental evidence of moderating effects at the community level. *Oecologia*, **117**, 177–186.

Docherty M, Wade FA, Hurst DK *et al*. (1997) Responses of tree sap-feeding herbivores to elevated CO_2. *Global Change Biology*, **3**, 51–59.

Douglas AE, Minto LB, Wilkinson TL (2001) Quantifying nutrient production by the microbial symbionts in an aphid. *Journal of Experimental Biology*, **204**, 349–358.

Fajer ED, Bowers MD, Bazzaz FA (1992) The effect of nutrients and enriched CO_2 environments on production of carbon-based allelochemicals in plantago – a test of the carbon nutrient balance hypothesis. *American Naturalist*, **140**, 707–723.

Flynn DFB, Sudderth EA, Bazzaz FA (2005) Effects of aphid herbivory on biomass and leaf-level physiology of *Solanum dulcamara* under elevated temperature and CO_2. *Environmental and Experimental Botany*, in press.

Goverde M, Arnone JA, Erhardt A (2002) Species-specific reactions to elevated CO_2 and nutrient availability in four grass species. *Basic and Applied Ecology*, **3**, 221–227.

Hattenschwiler S, Schafellner C (1999) Opposing effects of elevated CO_2 and N deposition on *Lymantria monacha* larvae feeding on spruce trees. *Oecologia*, **118**, 210–217.

Henn MW, Schopf R (2001) Response of beech (*Fagus sylvatica*) to elevated CO_2 and N: influence on larval performance of the gypsy moth *Lymantria dispar* (Lep., Lymantriidae). *Journal of Applied Entomology-Zeitschrift fur Angewandte Entomologie*, **125**, 501–505.

Hughes L, Bazzaz FA (2001) Effects of elevated CO_2 on five plant–aphid interactions. *Entomologia Experimentalis et Applicata*, **99**, 87–96.

Huhta AP, Hellstrom K, Rautio P *et al*. (2003) Grazing tolerance of *Gentianella amarella* and other monocarpic herbs: why is tolerance highest at low damage levels? *Plant Ecology*, **166**, 49–61.

Jansson J, Ekbon B (2002) The effect of different plant nutrient regimes on the aphid *Macrosiphum euphorbiae* growing on petunia. *Entomologia Experimentalis et Applicata*, **104**, 109–116.

Johnson RH, Lincoln DE (1991) Sagebrush carbon allocation patterns and grasshopper nutrition – the influence of CO_2 enrichment and soil mineral limitation. *Oecologia*, **87**, 127–134.

Karley AJ, Douglas AE, Parker WE (2002) Amino acid composition and nutritional quality of potato leaf phloem sap for aphids. *The Journal of Experimental Biology*, **205**, 3009–3018.

Kerslake JE, Woodin SJ, Hartley SE (1998) Effects of carbon dioxide and nitrogen enrichment on a plant–insect interaction: the quality of *Calluna vulgaris* as a host for *Operophtera brumata*. *New Phytologist*, **140**, 43–53.

Kinney KK, Lindroth RL, Jung SM *et al*. (1997) Effects of CO_2 and NO_{3^-} availability on deciduous trees: phytochemistry and insect performance. *Ecology*, **78**, 215–230.

Lalonde S, Tegeder M, Throne-Holst M *et al*. (2003) Phloem loading and unloading of sugars and amino acids. *Plant Cell and Environment*, **26**, 37–56.

Lawton JH (2000) *Community Ecology in a Changing World*. Ecology Institute, Luhe, Germany.

Mattson WJ Jr. (1982) Herbivory in relation to plant nitrogen content. *Annual Review of Ecology and Systematics*, **11**, 119–161.

Newman JA, Gibson DJ, Parsons AJ *et al*. (2003) How predictable are aphid population responses to elevated CO_2? *Journal of Animal Ecology*, **72**, 556–566.

Sandstrom J, Moran N (1999) How nutritionally imbalanced is phloem sap for aphids? *Entomologia Experimentalis et Applicata*, **91**, 203–210.

Sandstrom J, Moran N (2001) Amino acid budgets in three aphid species using the same host plant. *Physiological Entomology*, **26**, 202–211.

Saxon ME, Davis MA, Pritchard SG *et al*. (2004) Influence of elevated CO_2, nitrogen, and *Pinus elliottii* genotypes on performance of the redheaded pine sawfly, *Neodiprion lecontei*. *Canadian Journal of Forest Research – Revue Canadienne De Recherche Forestiere*, **34**, 1007–1017.

Vitousek PM, Mooney HA, Lubchenco J *et al*. (1997) Human domination of Earth's ecosystems. *Science*, **277**, 494–499.

Watt AD, Whittaker JB, Docherty M *et al*. (1993) The impact of elevated CO_2 on insect herbivores. In: *Insects in a Changing Environment* (ed. Harrington R, Stork NE), pp. 197–217. Academic Press, London.

Whittaker JB (1999) Impacts and responses at population level of herbivorous insects to elevated CO_2. *European Journal of Entomology*, **96**, 149–156.

Wigley TML, Raper SCB (2001) Interpretation of high projections for global-mean warming. *Science*, **293**, 451–454.

Wilkinson TL, Ishikawa H (2001) On the functional significance of symbiotic microorganisms in the Homoptera: a comparative study of *Acyrthosiphon pisum* and *Nilaparvata lugens*. *Physiological Entomology*, **26**, 86–93.